Output of lysosomal contents and cholesterol into bile can be stimulated by taurodehydrocholate

Khalid RAHMAN and Roger COLEMAN

Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

Although biliary secretion of phospholipids and cholesterol is principally dependent on bile-salt secretion, at low bile-salt output secretion of some lipids continues; also the amount of cholesterol secretion is more than that of phospholipid under these conditions, but the origin of this cholesterol is not known. Taurodehydrocholate was continuously infused in isolated perfused rat livers under recycling perfusion conditions and the biliary lysosomal output and lipid output measured. The rate of acid phosphatase and β -glucuronidase output increased 30–60 and 60–90 min respectively after liver isolation under these conditions. The rate of output of cholesterol and phospholipid increased in all the samples collected from taurodehydrocholate-infused livers. The increase in cholesterol output was approximately twice that of phospholipid output, leading to an increase in the cholesterol/phospholipid ratio in the bile.

INTRODUCTION

The greatest influence on both biliary cholesterol and phospholipid secretion is the secretion of bile salts; that is, with increasing bile-salt secretion there is increasing secretion of cholesterol and phospholipids (Hardison & Apter, 1972; Wheeler & King, 1972; Rahman *et al.*, 1986). When the hepatic influx of bile salts is low, as reported in man and in animals with an interrupted enterohepatic circulation (Thureborn, 1962; Balint *et al.*, 1971), there is an associated decrease in the secretion of biliary lipids. The effect on phospholipid secretion is greater than that on cholesterol secretion, and therefore, at very low bile-salt secretion rates, there remains a residual lipid output of relatively high cholesterol/phospholipid ratios (Rahman & Coleman, 1986a).

We have demonstrated this biliary secretion at low bile-salt-output rates in isolated perfused rat livers (Rahman & Coleman, 1986a) and have shown that the concentration and output of this cholesterol fraction decreases significantly in phalloidin-treated livers compared with controls, although there is no significant decrease in residual phospholipids; thus the secretion of residual cholesterol and phospholipids can be uncoupled from each other by the action of phalloidin. Hence, a proportion of cholesterol gets into bile independently of both bile salts and phospholipids. We have suggested that this bile-salt-independent cholesterol may be entering bile from lysosomes (Rahman & Coleman, 1986a).

Lysosomal enzymes are secreted co-ordinately into bile (La Russo & Fowler, 1979; Lopez del Pino & La Russo, 1981). Therefore any material sequestered in hepatocyte lysosomes could undergo biliary secretion. Such a lysosome-to-bile secretory pathway could be important in the secretion of a variety of intra- and extra-cellular constituents (e.g. metals, hormones, proteins and lipoproteins) known to be sequestered in, or degraded by, hepatocyte lysosomes (Sternleib & Goldfisher, 1976; Dunn *et al.*, 1980; Jones *et al.*, 1980). The possibility therefore also exists that this secretory route might be involved in the secretion of some of the biliary

cholesterol and phospholipid, since these have been reported to originate from a preformed pool of lipids (Turley & Dietschy, 1979; Cronholm *et al.*, 1983).

In earlier experiments we showed the hydrophilic non-micelle-forming bile-salt analogue taurodehydrocholate (TDHC) to increase the speed of delivery of lipids, probably by increasing the movement of vesicles to the canalicular membrane (Rahman & Coleman, 1986b), whence they can be removed by the action of the more hydrophobic bile salt taurocholate. In the experiments reported here, TDHC was continuously infused under recycling perfusion conditions in isolated perfused rat livers. Several biliary parameters were studied to see if secretion rates were affected either differentially, or in parallel, by this unusual potentiation of vesicular (and organelle?) movements.

MATERIALS AND METHODS

Materials

The following fine-grade chemicals were used: TDHC (Calbiochem-Behring Corp., Bishops Cleeve, Herts., U.K.), heparin (Weddel Pharmaceuticals, London E.C.1, U.K.) and Sagatal (May and Baker, Dagenham, Essex, U.K.). Hydroxysteroid dehydrogenase (grade II, from *Pseudomonas testosteroni*) was obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. All other reagents were from Fisons Ltd., Loughborough, Leics., U.K., and were of the highest grade available. The plastic cannula tubing (PP10 and PP30) was obtained from Portex, Hythe, Kent, U.K.

Methods

Male Wistar rats, weighing 250–280 g were used throughout; these had been maintained on a standard laboratory diet in a constant-temperature environment (22 °C) and under a constant 12 h-light/12 h-dark cycle. The livers were isolated under recycling conditions as described previously (see Rahman *et al.*, 1986). In some experiments, as soon as the livers were isolated, infusion of TDHC (dissolved in red-cell-free and albumin-free

Abbreviation used: TDHC, taurodehydrocholate.

perfusion medium) into the perfusion line was initiated at a rate of 12 ml/h (1000 nmol/min per animal) and continued for 90 min.

Bile was collected in pre-weighed tubes on ice at 30 min intervals for 90 min. After completion of the perfusion the livers were weighed. The volume of bile was determined gravimetrically, a density of 1 g·ml⁻¹ being assumed. All bile samples and homogenates were stored at -20 °C until required for analysis.

Assays

The following enzyme assays were used: acid phosphatase (EC 3.1.3.2) (Hübscher & West, 1965); β -D-glucuronidase (EC 3.2.1.31) (Gianetto & de Duve, 1955). The enzymes were assayed at 37 °C under conditions where activity was proportional to protein concentration; suitable enzyme and substrate blanks were incorporated in all assays. Activities are expressed as μ mol of substrate hydrolysed/h per ml of bile and as munits / min (1 munit = 1 mmol of substrate hydrolysed/h) respectively.

Bile was assayed for phospholipid after lipid extraction by the method of Bligh & Dyer (1959), with phospholipid-phosphorus determination by the method of Bartlett (1959). Bile-salt concentrations were determined with hydroxysteroid dehydrogenase (EC 1.1.1.50) as described by Coleman *et al.* (1979).

Analysis of biliary cholesterol was performed as described previously (Rahman & Coleman, 1986a).

RESULTS

After isolation and perfusion of the liver, bile was collected for three 30-min intervals from both control and TDHC-infused livers. Bile flow decreased with respect to time in the control livers, largely owing to a decrease in bile-salt-dependent flow occasioned by loss of bile salts (see below). The 60–90 min fraction is mainly a bile-salt-independent flow. In the TDHC-infused livers the increased in bile flow in the 0–30 min sample was twice that observed in control livers; thereafter it was three times the control values, owing to the choleric effect of the TDHC (Table 1).

Bile-salt output decreased in control livers with time, owing to interruption of the enterohepatic circulation, and in the 60–90 min fraction probably represented bile salts principally from synthesis *de novo*. The bile-salt (measured by steroid dehydrogenase) output in TDHC-infused livers remained constantly high during the experiment; this is probably due to the presence of the major derivatives of this bile-salt analogue, which are assayed by steroid dehydrogenase (O'Maille & Hofmann, 1986).

The rate of output of acid phosphatase (munits/min) was similar after 30 min in bile from both control and TDHC-infused livers, but was significantly increased, however, in the 30–60 and 60–90 min samples from TDHC-infused livers (Table 1). The activity of acid phosphatase (μ mol/h per ml) was similar up to 60 min in both control and TDHC-infused livers, but it was significantly decreased in the 60–90 min sample from TDHC-infused livers owing to the volume increase of bile (Table 1). Similar phenomena were observed for β -glucuronidase. The rate of output (munits/min) of β -glucuronidase stayed relatively the same up to 30 min in control and TDHC-infused livers, but was significantly

increased in the 30–60 and 60–90 min samples from TDHC-infused livers (Table 1). The activity of β -glucuronidase (μ mol/h per ml) was similar up to 60 min in both control and TDHC-infused livers, but it was significantly decreased in the 60–90 min sample from TDHC-infused livers, again due to the volume increase of bile (Table 1).

Cholesterol-output rate in bile decreased with bile-salt secretion. The cholesterol concentration also decreased in approximately the same manner (Table 1). In TDHC-infused livers, however, the total cholesterol output was significantly increased in all the samples, and its concentration increased in the 30–60 and 60–90 min samples (Table 1).

In control livers both biliary phospholipid output and concentration decreased with decreasing bile-salt output. In TDHC-infused livers the phospholipid output was significantly increased in all the samples, but the biliary phospholipid concentration only increased significantly in the 60–90 min sample (Table 1).

The increase in cholesterol secretion was greater than that of phospholipid secretion under these conditions, leading to an increase in the cholesterol/phospholipid ratio in the bile from TDHC-infused livers (Table 1).

DISCUSSION

In control livers the bile flow decreased, owing to decreasing bile-salt output, and in the 60–90 min sample the bile flow is mainly bile-salt-independent. The increase in bile flow in TDHC-infused livers was twice that of control values in the 0–30 min sample, and thereafter it increased to three times the control values (Table 1). The leading theory for the formation of canalicular bile is that of local osmosis, i.e. that solute is transported into the bile canaliculus, with fluid following secondarily along an osmotic gradient. The finding that TDHC increases bile flow is therefore expected, as it has been reported that there is no significant decrease in osmotic activity by micellar aggregation of 'dehydrocholate' in bile (O'Maille & Richards, 1976).

It has been reported that, during TDHC administration in the fistula rat, the major derivatives which appear in bile are 3 α -hydroxy-7,12-dioxocholanoyletaurine and 3 α ,12 α -dihydroxy-7-oxocholanoyletaurine (O'Maille & Hofmann, 1986). Hence the 3 α -hydroxy-bile-acid concentration in bile (assayed by steroid dehydrogenase) gives a satisfactory estimate of the total bile-acid concentration in bile during TDHC infusion (O'Maille & Hofmann, 1986). Studies *in vitro* have also shown that the major derivative of TDHC, namely 3 α -hydroxy-7,12-dioxocholanoate, has a critical micellar concentration of more than 50 mM (Roda *et al.*, 1983). The concentration of TDHC derivatives in bile from TDHC-infused livers was approx. 50 mM as assayed by steroid dehydrogenase (Table 1), i.e. below the critical micellar concentration. The major derivative of TDHC on its own also does not appear to solubilize any phospholipid (Gerolami *et al.*, 1972; Soloway *et al.*, 1973).

Compared with control values the rate of output of acid phosphatase (munits/min) was significantly increased in the 30–60 and 60–90 min samples from TDHC-infused livers (Table 1). In contrast, the activity of acid phosphatase (μ mol/h per ml) decreased significantly in the 60–90 min sample from TDHC-infused liver, owing to the increase in bile flow (Table 1). Similarly, the rate

Table 1. Effect of continuous TDHC infusion on biliary output in the isolated perfused rat liver

Bile was collected after liver isolation at 30 min intervals up to 90 min. TDHC was continuously infused at a rate of 1000 nmol/min under recycling perfusion conditions. Values are means $\pm$ S.E.M. for the number of observations given; significant differences from controls are indicated by * ($P < 0.05$).

Parameter	Control (<i>n</i> = 5)			TDHC (<i>n</i> = 3)			
	Time of bile collection (min) . . .	0-30	30-60	60-90	0-30	30-60	60-90
Bile (μl/min)		18.5 ± 0.5	11.9 ± 0.6	8.8 ± 0.4	35.0 ± 0.7*	32.5 ± 0.9*	28.7 ± 1.0*
Bile salt							
Concn. (mM)		3.15 ± 0.48	2.42 ± 0.21	2.47 ± 0.19	45.00 ± 2.45	51.59 ± 0.85	51.70 ± 3.15
Output (nmol/min)		58.6 ± 10.2	29.1 ± 2.4	21.9 ± 2.4	1573 ± 68	1676 ± 64	1480 ± 73
Acid phosphatase							
munits/min		29.0 ± 0	21.0 ± 0	20.0 ± 0	45.0 ± 0	45.0 ± 0*	44.0 ± 0*
μmol/h per ml		1.58 ± 0.16	1.81 ± 0.22	2.29 ± 0.10	1.29 ± 0.22	1.40 ± 0.03	1.01 ± 0.51*
β-Glucuronidase							
munits/min		3.6 ± 0	3.8 ± 0	4.0 ± 0	3.6 ± 0	7.3 ± 0.0*	8.1 ± 0*
μmol/h per ml		0.19 ± 0.02	0.33 ± 0.07	0.46 ± 0.06	0.10 ± 0.04	0.16 ± 0.08	0.28 ± 0.04*
Cholesterol							
Output (nmol/min)		1.59 ± 0.39	0.46 ± 0.16	0.39 ± 0.09	3.96 ± 0.55*	3.09 ± 0.27*	3.26 ± 0.27*
Concn. (mM)		0.086 ± 0.020	0.038 ± 0.010	0.45 ± 0.010	0.113 ± 0.20	0.096 ± 0.10*	0.113 ± 0.010*
Phospholipid							
Output (nmol/min)		31.34 ± 5.60	7.43 ± 1.57	5.31 ± 1.39	50.5 ± 3.1*	34.7 ± 4.2*	34.6 ± 2.8*
Concn. (mM)		1.69 ± 0.28	0.62 ± 0.12	0.60 ± 0.15	1.45 ± 0.1	1.07 ± 0.15	1.21 ± 0.13*
Cholesterol/phospholipid ratio (mol/mol)							
Liver weight (g)		0.051	0.061	0.075	0.078	0.090	0.093
			10.82 ± 0.17			10.12 ± 0.32	

of output of β -glucuronidase (munits/min) was significantly increase in the 30–60 and 60–90 min samples from TDHC-infused livers (Table 1). The activity of β -glucuronidase (μ mol/h per ml) decreased significantly in the 60–90 min sample from TDHC-infused livers, owing to the increase in bile flow (Table 1). Thus it appears that TDHC and its metabolites stimulate the rate of movement of lysosomes to the canalicular membrane and their fusion with it. This would result in an increase in lysosomal contents appearing in bile, as evidenced by an increase in lysosomal-enzyme output (Table 1).

TDHC infusion increased phospholipid output by 0.6-fold at 0–30 min, 3.5-fold at 30–60 min and 5.5-fold at 60–90 min when compared with control values (Table 1). In similar experiments a comparable infusion of taurocholate which achieved the concentration of 50 mM resulted in an increase of phospholipid output of approx. 135 nmol/min (K. Rahman, unpublished work). In the 60–90 min sample from TDHC-infused livers the increase in phospholipid output is 29.3 nmol/min (Table 1). Therefore TDHC and its metabolites increase biliary phospholipid output by approx. 22% and thus do not appear to be as efficient as taurocholate in stimulating biliary lipid output. This is in agreement with the observation reported by Barnwell *et al.* (1984).

However, TDHC and its metabolites increased cholesterol output by a much greater extent than they did that of phospholipid. Cholesterol output was increased by 1.5-fold at 0.30 min, 6-fold at 30–60 min and 7.5-fold at 60–90 min in the samples from TDHC-infused livers when compared with control values (Table 1). In the 60–90 min sample from TDHC-infused liver there was an increase in cholesterol output of 2.87 nmol/min when compared with the similar sample from control liver (Table 1). Comparable taurocholate perfusion increased cholesterol output by 6.5 nmol/min (K. Rahman, unpublished work). Therefore TDHC and its metabolites stimulate biliary cholesterol by approx. 45% compared with similar amounts of taurocholate. A small stimulation of biliary cholesterol secretion by TDHC has also been reported by Barnwell *et al.* (1984). Hence TDHC appears to be twice as efficient in stimulating biliary cholesterol as in stimulating phospholipid, and this implies that both lipids, besides sharing a common route, might be getting into bile by two separate mechanisms. This differential secretion of cholesterol and phospholipid is reflected in the higher cholesterol/phospholipid ratios present in samples from isolated perfused livers infused with TDHC (Table 1).

It is clear from the data that TDHC infusion brings about an increase in both lysosomal material and lipids in bile (Table 1). The origin of the increases in lipid secretion is not yet clear. A proportion of the lipid increase (i.e. 0–30 min) precedes the increased lysosomal-enzyme secretion (30–90 min), and the subsequently lipid increase (30–90 min) is coincident. Whether the lysosomal material entering the bile is directly responsible for the (30–90 min) cholesterol-rich lipid fractions is not possible to tell from the data. La Russo *et al.* (1982), using Triton WR-1339, a lysosomotropic compound, have shown that lysosomal enzymes in bile are increased, together with phospholipid, but not cholesterol. In contrast, O'Maille & Hofmann (1986) have shown that, in the fistula rat, infusion of TDHC did not increase

biliary phospholipid and cholesterol levels above basal values. Owing to the relatively small increase in total lipid output in TDHC infusion reported here, it is possible that those authors did not see an increase in lipid output, as their background biliary lipid concentration would be higher in the whole animal.

Besides stimulating lysosomal-enzyme output, TDHC and its metabolites may be stimulating the movement of vesicles carrying lipids to the canalicular membrane (see Rahman & Coleman, 1986b). The membrane of these, or their contents, may be released into bile at a low rate, but require a more hydrophobic bile salt for maximum output into bile, and would thus account for the non-lysosomal biliary lipid output.

We thank the M.R.C. (Medical Research Council) for financial assistance, K.R. is in receipt of an M.R.C. Research Fellowship.

REFERENCES

- Balint, J. A., Beeler, D. A., Kyriakides, E. C. & Treble, D. H. (1971) *J. Lab. Clin. Med.* **77**, 122–123
- Barnwell, S. G., Lowe, P. J. & Coleman, R. (1984) *Biochem. J.* **220**, 723–731
- Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–919
- Coleman, R., Iqbal, S., Godfrey, P. P. & Billington, D. (1979) *Biochem. J.* **178**, 201–208
- Cronholm, T., Curstedt, T. & Sjovall, J. (1983) *Biochim. Biophys. Acta* **753**, 276–279
- Dunn, W. A., Hubbard, A. L. & Aronson, N. N. (1980) *J. Biol. Chem.* **255**, 4971–4978
- Gerolami, A., Crott, C., Montet, J. C., Vigne, J. L., Granger, M. & Mule, A. (1972) *Biol. Gastro-Enterol.* **5**, 265–272
- Gianetto, R. & de Duve, C. (1955) *Biochem. J.* **59**, 433–438
- Hardison, W. G. M. & Apter, J. T. (1972) *Am. J. Physiol.* **22**, 61–67
- Hübscher, G. & West, G. R. (1965) *Nature (London)* **205**, 799–800
- Jones, A. L., Schmucker, D. A., Renston, R. H. & Murakami, T. (1980) *Dig. Dis. Sci.* **25**, 609–629
- La Russo, N. F. & Fowler, S. (1979) *J. Clin. Invest.* **64**, 948–954
- La Russo, N. F., Kost, L. J., Carter, J. A. & Barham, S. S. (1982) *Hepatology* **2**, 209–215
- Lopez del Pino, V. & La Russo, N. F. (1981) *J. Lipid Res.* **22**, 229–235
- O'Maille, E. R. L. & Richards, T. G. (1976) *J. Physiol. (London)* **261**, 337–357
- O'Maille, E. R. L. & Hofmann, A. F. (1986) *Q. J. Exp. Physiol.* **71**, 475–482
- Rahman, K. & Coleman, R. (1986a) *Biochem. J.* **237**, 301–304
- Rahman, K. & Coleman, R. (1986b) *Enterohepatic Circulation of Bile Acids and Sterol Metabolism*, MTP Press, Lancaster (U.K.), in the press
- Rahman, K., Hammond, T. G., Lowe, P. J., Barnwell, S. G., Clark, B. & Coleman, R. (1986) *Biochem. J.* **234**, 421–427
- Roda, A., Hofmann, A. F. & Mysels, K. J. (1983) *J. Biol. Chem.* **258**, 6362–6370
- Soloway, R. D., Hofmann, A. F., Thomas, P. J., Schoenfield, L. J. & Klein, P. D. (1973) *J. Clin. Invest.* **52**, 715–724
- Sternleib, I. & Goldfisher, S. (1976) in *Lysosomes in Biology and Pathology* (Dingle, J. T. & Dean, R. T., eds.), vol. 5, pp. 185–202, North-Holland Publishing Co., Amsterdam
- Thureborn, E. (1962) *Acta Chir. Scand. Suppl.* **303**, 3–63
- Turley, S. D. & Dietschy, J. M. (1979) *J. Lipid Res.* **20**, 923–934
- Wheeler, H. O. & King, K. K. (1972) *J. Clin. Invest.* **511**, 1337–1345